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Hemolytic streptococci on the body surface

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HEMOLYTIC STREPTOCOCCI ON THE BODY SURFACE

BY

JOSEPH ANDREW SHACTER

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THESIS

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July 1, 1919

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY
SUPERVISION BY Joseph Andrew Shacter
ENTITLED Hemolytic Streptococci on the Body Surface

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR
THE DEGREE OF Master of Science

D. J. Davis

In Charge of Thesis

D. J. Davis

Head of Department

Recommendation concurred in*

Committee
on
Final Examination*

*Required for doctor's degree but not for master's

Table of Contents

- I Introduction
- II Literature
- III Hemolytic Streptococci on the Normal Skin.
 - (a) Streptococci in the "clean group".
 - (b) Streptococci in the "filthy group".
 - (c) Fermentation reaction of isolated streptococci (Table 1.)
 - (d) Pathogenicity of isolated streptococci.
 - (e) Viability of isolated streptococci on normal skin.
 - (f) Corroborative data.
- IV Bacterial Content (Streptococcus Hemolyticus) of the
Deeper Layers of the Skin.
 - (a) Literature.
 - (b) Experimental Study.
 - (c) Sources of material (Table 2.)
 - (d) Results and Conclusions.
- V Do Hemolytic Streptococci Occur in Various Skin Lesions?
 - (a) Experimental Study.
 - (b) Sources of material (Table 3.)

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(c) Results and Conclusions.

VI Summary.

VII Bibliography.

Hemolytic streptococci have been found to play an important etiologic role in a great variety of infections, both in civil and in military life. The relationship of this virulent organism to different pathological conditions has been carefully studied by many workers from a number of different viewpoints. Diseases of the respiratory tract, for example, have often been due to this organism, together with the enormous number of pulmonary complications resulting from its invasion. In war wounds it has been found to be the most troublesome and the most dangerous organism to deal with, both as to the infection per se, and to subsequent wound closure and suture.

In this paper I will present the results of certain bacteriological experimental work with the streptococcus hemolyticus in relation to the skin and its deeper layers, both in normal and in certain pathological conditions.

In reviewing the literature, little work has been met with, giving experimental data on the subject of hemolytic streptococci in relation to the skin of the human body. Weaver (1.) obtained streptococci in small numbers from scales taken from the skin of the abdomen in a single case of scarlatina. He intimates that they probably had been transferred from the mouth to the skin. In this early work, differentiation into hemolytic and nonhemolytic was not determined. Raskin (2.) found streptococci on the scales 4 times in 20 observations, but never in the intact skin. Gordon's (3.) examinations of the skin for streptococcus were negative. Chipman (4.) recently remarks that "the streptococcus often, but by no means always, evinces a selective preference for certain sites such as the folds of the skin, as in the axillary region." He neither refers to nor offers experimental data to verify this statement.

Reference need only be made to standard textbooks on surgery and dermatology to ascertain the important etiologic role played by the hemolytic streptococcus in its relation to the skin. Erysipelas is of the greatest importance in this regard, the hemolytic strepto-

5.
coccus invading the skin, and being responsible for the production of this infection. Suffice it to merely mention also the importance of this organism in various surgical complications of war wounds, often leading to most serious results.

As a matter of prophylaxis, it is evidently of importance to know how commonly hemolytic streptococci are present on the skin of soldiers before the occurrence of wounds. Alexander (5.) has demonstrated that the hemolytic streptococcus is present in the dust, atmosphere, and floor sweepings of the quarters housing soldiers; there is, of course, the possibility of their presence in the soil, water, etc., and in the various vehicles used to transport the soldiers to the evacuating stations and base hospitals, and also their presence on clothing.

Hemolytic Streptococci on the Normal Skin.

(a) Experimental Study.

In order to make a comparative survey of the surface of the body for hemolytic streptococci, a topographical outline was arranged corresponding much to the regiones corpores humane of Spalteholz. This plan, therefore, included all of the exposed sites of the body surface, especial reference being given, however, to the head and hands, since these parts are practically always exposed. Cultures were taken with the ordinary platinum wire loop, first dipped in sterile water to insure adhesion between the organism and the loop. With a sterile scalpel the skin area was first scraped, in order that the deeper layers of the skin might be reached, and with a moderate amount of pressure the wire loop containing the sterile water was then applied. Cultures were made on blood agar plates and then incubated for 24 hours at 37^o C. Hemolytic streptococci, when present, grew typically as small, round, gray, raised colonies, with a wide, clear zone of hemolysis around each colony. The suspicious streptococci appearing on the plates were then studied morphologically and culturally. Pathogenicity, sugar fermentations, and other properties were also determined, and will

be detailed later.

III (b)

Streptococci in the "Clean Group."

In the first experiments, cultures were taken from 38 persons, all of whom, on inquiry, stated that they bathed at least once a week, and several said that they took shower baths every morning. The cultures were taken from the same areas on each body, in order to insure a comparative study. From this group, which may be called a "clean group," no hemolytic streptococcus colonies were isolated in any case. The organisms found were, as a rule, white staphylococci.

III (c)

Streptococci in the "Filthy Group."

A second group, numbering 89 individuals selected largely from dispensary patients, and, as a rule, very filthy in their habits, were next examined in the same way as the first group. The results are as follows: From these 89 cases 15 strains of hemolytic streptococci were isolated from 8 different persons, thus giving 9 per cent of the "filthy group" from which the organisms were isolated. Ten of the 15 strains of hemolytic streptococci

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were obtained from various regions of the head, 5 of which came from hairy parts. One colony was isolated from the tibia, which was thickly covered with hair. One colony was isolated from the hands. This gives 73 per cent of the organisms isolated from the exposed parts of the body, the other organisms being obtained, as previously noted, from parts ordinarily covered by clothing.

III (d)

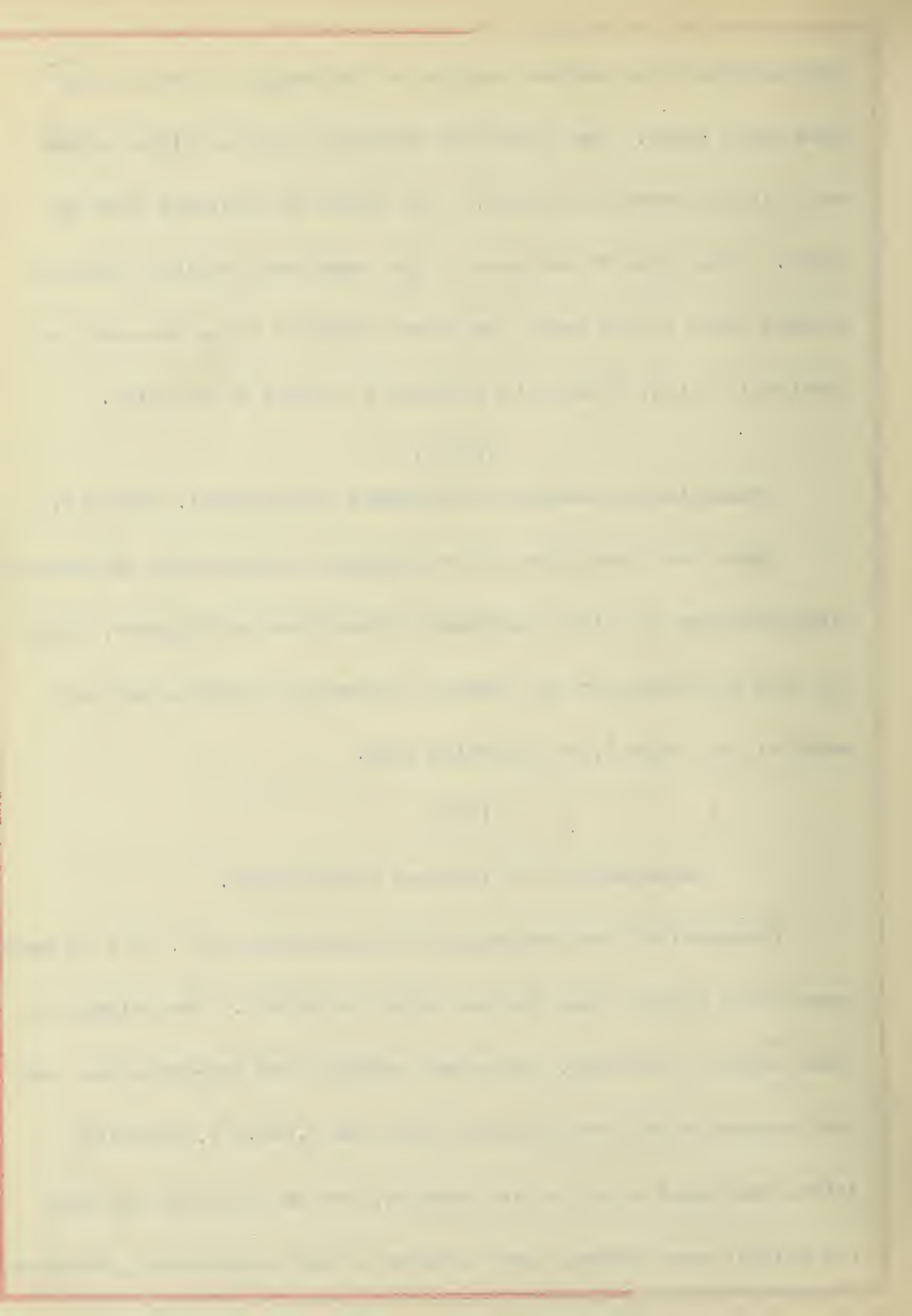
Fermentation Reaction of Isolated Streptococci. (Table 1.)

Sugar reactions place these organisms on the basis of Holman's classification (7.) into the group, "Streptococcus Pyogenes." That is, they are hemolytic and ferment lactose and salicin, but not mannite. See table 1, on following page.

III (e)

Pathogenicity of Isolated Streptococci.

Pathogenicity was determined by inoculating 2 ml. of a 24 hour serum broth culture into the ear vein of a rabbit. The weights of these animals decreased, the amount varying from 150-300 grams, and the temperature in each instance rose from 0.5-2.5^o F. Arthritic joints developed in all of the rabbits, and on the tenth day when the animals were posted, pure cultures of the streptococci, identical



Fermentation Reaction of Isolated Streptococci.

Table 1.

Streptococcus Hemolyticus

Gram Positive Cocci in Chains, No Capsules.

Rabbit	Lactose	Mannit	Salicin	Glucose	Saccharose
1	+	—	+	+	+
2	+	—	+	+	+
3	+	—	+	+	+
4	+	—	+	+	+
5	+	—	+	+	+
6	+	—	+	+	+
7	+	—	+	+	+
8	+	—	+	+	+
9	+	—	+	+	+
10	+	—	+	+	+
11	+	—	+	+	+
12	+	—	+	+	+
13	+	—	+	+	+
14	+	—	+	+	+
15	+	—	+	+	+

9.

with those injected, were isolated from each of the swollen joints. One of the rabbits died on the sixth day, and showed a marked acute fibrinous pericarditis, pure cultures of the organism being obtained from the heart blood. Three of the rabbits showed little effect of the first injection, and required reinoculation on the fifteenth day. Subsequently they lost on the average 130 gm. in weight, and a rise in temperature of 1.5^o F., and developed typical arthritic joints, from which hemolytic streptococci were isolated in pure culture.

III (f)

Viability of Isolated Streptococci on the Normal Skin.

To ascertain the probable duration of these strains on the skin, a loopful of a 24 hour serum-broth culture of a typical hemolytic streptococcus was rubbed into the skin with the wire loop at three points on the intact skin, and at three sites on the surface scraped with a sterile scalpel on the dorsum of the left hand. For a period of 34 hours the hand was not washed or wiped, and came in contact with clothing, only so far as was necessitated by their removal on retiring. Precisely the same experiments were performed

at the same time on the right hand, which was washed with soap and water several times during the day. Cultures were taken each ten minutes for the first hour, and each half hour for the succeeding thirty three hours.

The colonies at but one site, on the right hand, survived the first washing, which was four hours after the experiment was inaugurated. This was on the scraped skin. One and one-half hours later cultures from this area were negative.

On the left hand, two of the zones in the intact skin revealed colonies up to the tenth hour, the third site gave a positive culture a half hour longer. In the scraped skin, one area was negative at the twelfth hour, the other two sites still giving positive results. At this hour, 8 p. m., I left the laboratory. At 8:05 a. m., the following morning, only one of the areas of the scraped skin yielded a culture of the organism. This site was negative at 10:30 a. m. These determinations were repeated on another occasion, and found, in the main, to be the same.

Recently, and since this work was under way, confirmatory reports have been made by Lieut.-Col. J. G. Cumming. (8.) His

11.

investigation discloses the fact that of the hands of hemolytic streptococci carriers, 37 % were positive for this organism. Our work disclosed that in individuals who were not hemolytic streptococci carriers, about 1 % gave positive results from the hands; and in 8 % the same organism was found on other sites of the body surface, whereas in streptococcus carriers about 50 % (9.) were found to give positive cultures from the hands. Cumming found that the hands of a carrier were still positive after washing in soap and running water for seven minutes. As stated above, similar experiments performed by me, revealed that after intermittent washing with soap and water, cultures gave positive results four hours after the experiment was inaugurated. In general, the work of Cumming confirms the data presented in this paper.

From the data obtained in this work, no colonies of hemolytic streptococci were isolated from the sites mentioned by Chipman, notwithstanding the fact that especial care and attention were given in making cultures from these zones.

Cultures taken from underneath the finger nails were in all instances negative for hemolytic streptococci, but contained many

white staphylococci and other bacteria.

From finding hemolytic streptococci in the series of cultures made by Malone and Rhea (6.) and others from war wounds, it is easy to presume that in at least a certain percentage of cases, the hemolytic streptococcus is present as either a primary or a secondary invader, the organism being present on the skin previous to the occurrence of the wound, and starting mischief only when a better food medium presented itself.

Deeper Layers of the Skin.

(a)

Recently numerous papers have been written concerning the presence of hemolytic streptococci in wounds of war and in clean surgical wounds in military hospitals both in Europe and in America. Blanton, Burhans, and Hunter (10.) found a number of wounds infected with hemolytic streptococci in a clean surgical ward at Camp Custer. They concluded after careful observation that the probable source of the infection was most probably the mouth, the contamination occurring either as a transient symptomless bacteremia, or transfer of the organisms from mouth to wound by means of the hands. Keegan (11.) similarly found in clean surgical cases, a deep infection of wounds with hemolytic streptococci. Rigid bacteriologic examination disclosed the fact that the throats and hands of the operating room staff were negative for hemolytic streptococci, as were cultures from catgut and clean wounds before closure. Keegan points out that careful analysis of the surgical case histories showed that every case of wound infection with the hemolytic streptococcus was preceded for two, three, or four days by a sore throat,

and a sudden rise of temperature, with normal or nearly normal temperature up to the time of the appearance of wound symptoms.

Bunce, Berlin, and Lawrence (12.) found hemolytic streptococci in twenty four per cent of war wounds. They conclude that the presence of this organism is the chief cause of the failure of secondary suture, and that therefore no wound showing a positive culture of streptococcus hemolyticus should be closed. The probable etiology of these hemolytic streptococci in war wounds, was not apparently investigated, since the authors make no mention as to their possible source.

On the basis of the above findings by me, the reports of Malone and Rhea, and Bunce, Berlin, and Lawrence, as to the presence of hemolytic streptococci in war wounds, can to some extent at least be readily explained. The work of Blanton et. al., and Keegan, however, suggests the possibility of another avenue of infection, namely, the deeper layers of the skin, where hemolytic streptococci might be harbored, and be practically unaffected by soap and water, or the application of iodine and other antiseptics previous to operation.

Experimental Study.

An investigation was undertaken to determine the possible presence of these organisms in the deeper as well as in the superficial layers of the skin, so that one of the causes of wound infection, whether accidental or operative, might be ascertained or ruled out. Material was gathered chiefly from surgical operations and post mortem tissue. The tissue was collected in bottles or containers partly filled with sterile salt solution. A diagnosis of the surgical procedure was given in each instance in order to rule out the possibility of streptococcus infections of various kinds.

The tissue surface was first carefully cultured on blood agar with a platinum wire loop and sterile cotton swabs, to determine the presence or absence of hemolytic streptococci. The following steps were then followed as outlined in the main by Rosenow. (13.) The surface of the part used for cultures is sterilized by dipping it into boiling water, or into a vigorous Bunsen flame, or seared with a hot blade; then rapidly cooled in sterile salt solution and emulsified in the specially devised sterile air chamber or "tissue

16.

crusher." Instead of using the meat chopper, the older type of crushing can was used as outlined by the same author. (14.) In the early experiments it was found that using the meat grinding device resulted in the loss of a variable amount of tissue, which remained within the instrument, or on the inner side of the blades, making aseptic removal almost impossible.

Following a thorough crushing of the tissue in a mortar within the crushing can, with the aid of sterile sand and the addition of sterile broth, the resulting emulsion was poured into tubes containing sterile melted blood agar. The entire mixture was then well shaken and poured into sterile plates, which were incubated at 37 C. for 24-72 hours, under aerobic conditions. Rigid observation was made as to the presence of hemolyzing colonies; other colonies occurring on the plates were studied culturally and by stained smear.

The data obtained was gathered from the following sources, as detailed in table on following page:

Sources of Material (Table 2.)

Skin from Abdominal Wall	7
Skin from Circumcisions (adults 19)	22
Skin from Amputations:	
Fingers	34
Forearm	2
Upper Arm	1
Leg	4
Thigh	2
Post Mortem (24 hour)	7
Post Mortem (96 hour)	11

The tissue obtained at necropsy was taken from the scalp, abdomen, and chest, in the routine of brain and viscera removal.

IV (d)

Results and Conclusions.

According to the size of the tissue, three to eleven plates were made in each case. Altogether, 95 pieces of tissue were cultured as described. In not one instance was the hemolytic streptococcus isolated. Usually the plates were practically sterile;

occasionally a colony or two of staphylococcus albus was observed.

Practically no other organisms appeared.

From the results obtained, it seem evident that hemolytic streptococci normally do not invade the skin or live in the deeper layers. Some defensive mechanism of the body is no doubt present, which serves to ward off the invasion of the deeper layers of the skin by microorganisms, and prevent their development there. The most probable mechanism of such protection is the presence of leucocytes.

(a)

Experimental Study.

Having previously determined the presence of the hemolytic streptococcus on so-called "dirty" skin surfaces, the question as to whether these organisms are present in skin lesions next suggested itself. Especially in long standing chronic cases, of the dispensary type, would one be led to suspect their presence.

In gathering material for this investigation, it was aimed to make cultures from those cases which were never treated at any previous time. In many instances patients may have had local treatment in the past, but when presenting themselves at the dispensary, their dermatologic lesions were not subjected to treatment as yet. Cultures were taken systematically, following a routine method previously planned. A sterile cotton swab, first dipped into sterile salt solution, was passed over the affected skin, pressure being used over the entire surface. This swab was then streaked over a freshly prepared blood agar plate. Several drops of fresh sterile blood were placed on the already hardened blood agar plate, the swab being passed thru these and evenly distributed over the entire

plate. Next, a sterile wire loop was passed over the surface of the lesion, be it a macule, papule, vesicle, or pustule. As previously mentioned, sufficient pressure was used to insure thorough contact of the platinum wire loop with the skin surface. The cultures thus gathered, were then inoculated into freshly prepared tubes of blood agar, and poured into sterile plates.

The cultures from these skin surfaces revealed very numerous colonies of white staphylococci, and occasionally a few colonies of green streptococci. Hemolytic streptococci were absent in all cases save two. In both of these cases (acnea rosacea and psoriasis) the individuals were suffering with sore throats, from which hemolytic streptococci were isolated. These organisms were of the typical hemolytic variety. In the three cases of scarlet fever, cultures from the hands, face, and trunk, revealed no hemolytic streptococci, altho streptococci of the green variety were obtained.

The next step was to analyse the contents of the lesion per se. With a sterile scalpel and forceps, the cover of the lesion carefully removed and cultures taken from the internal lining and contents of said lesion. The material was then inoculated into

freshly prepared blood agar tubes, thoroughly shaken, and then plated and incubated in the usual manner.

The appended table gives the source of culture material.

V (b)

Sources of Material (Table 3.)

Psoriasis	31 Cases
Lichen Planus	3
Measles	4
Herpes Zoster	3
Pemphigus Neonatorum	1
Eczema	35
Acnea Rosacea	8
Furuncles	28
Pustular Syphilide	3
Blastomycosis	1
Sycosis	2
Scarlet Fever	3
Acnea Vulgaris	40
Impetigo Contagiosa	2
Actinomycosis	1

Results and Conclusions.

In all, fifteen different skin lesions were studied in 165 individuals. In not one instance was the hemolytic streptococcus isolated from any of the skin lesions referred to. Often the contents of the lesions were absolutely sterile, occasionally white staphylococci were isolated, this being especially true in furuncles.

From the above data it would appear that secondary infections with hemolytic streptococci do not as a rule occur in the skin. This fact has been found true in the routine of making examinations in collecting data for this paper. From the work of Unna (15.) one would be led to draw the conclusion that the protective mechanism of the body against the organisms in chronic lesions of the skin, was the massive numbers of leucocytes, as found for example in various eczematous lesions, even so far up in the skin layers as the horny stratum. In a case of chronic eczema, the same author found that there was a "saturation of leucocytes" dilating the uppermost layers of the skin into vesicles. In another case he found that the cover of the vesicle consisted of the horny layer of the skin, the contents of the vesicle being about one quarter leucocytes.

1. The data presented, indicates the prophylactic value of cleanliness on the streptococcus flora of the body surface.
2. In the "filthy group" composed of 89 cases, 9% revealed the presence of the hemolytic streptococcus on the skin of the body surface, whereas in the "clean group" of 38, not a single colony was isolated.
3. Of the 10 colonies isolated from the head, 5 were from hairy parts, the strain isolated from the tibia came from a region profusely covered with hair. This indicates that on the skin, hemolytic streptococci incline more to the hairy parts than they do to the skin surfaces.
4. These streptococci isolated from the skin are distinctly pathogenic for rabbits, and behave in this animal quite like ordinary pathogenic streptococci from other sources.
5. These streptococci appear to be typical hemolyzers, and on the basis of sugar reaction may be classed according to Holman, as "streptococcus pyogenes". The name indicates their pyogenic character, they being the hemolytic streptococci commonly causing purulent

purulent infections.

6. The deeper layers of the skin in a series of examinations of 95 different sections of tissue, gave negative cultures for hemolytic streptococci.

7. The hemolytic streptococcus, as indicated by the above bacteriologic tests, was not found in a series of 15 different skin lesions, occurring in 165 different individuals, as detailed in Table 2.

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